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SARS-COV-2 Triggers the Development of Class I and Class II HLA Antibodies in Recovered Convalescent Plasma Donors

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Keywords
SARS-CoV-2 · COVID-19 · HLA antibodies · Immune response · Convalescent plasma

Abstract
Various studies have shown that SARS-CoV-2 is a highly immunogenic virus. It is known that different types of immunogenic viral pathogens could trigger the formation of HLA antibodies. Therefore, there is a concern that the SARS-CoV-2 could also induce the development of HLA antibodies in volunteers, who donate convalescent plasma after their recovery from COVID-19. HLA antibodies have been identified as the main cause for transfusion-related acute lung injury (TRALI), a well-documented life-threatening complication of transfusions. The TRALI risk could be high in COVID-19 patients who need convalescent plasma, as such patients usually have already an impaired respiratory system affected by the SARS-CoV-2 infection. In this study, we screened 34 convalescent plasma donors on the presence of antibodies against HLA class I and II antigens. All included donors have no any history of sensitization events such as blood transfusions, pregnancy, or previous transplants. We found a high rate of HLA antibody formation in convalescent plasma donors. The frequency of positivity for HLA antibodies for class I, class II, class I and II, and the overall reactivity was 23%, 31%, 46%, and 76%, respectively. The presented data suggest a closed correlation between SARS-CoV-2 virus infection and the development of HLA antibodies in recovered convalescent plasma donors. This finding might have the potential to reduce the risk of TRALI and mortality rate in COVID-19 patients by implementing HLA diagnostic strategies before the administration of convalescent plasma.

Introduction

There is evidence that the formation of human leucocyte antibodies (HLA-Abs) can be triggered by different types of viral pathogens [1, 2]. It would be interesting to know whether SARS-CoV-2 virus is able to induce HLA

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antibodies in infected persons. An important goal of this study was therefore to determine whether the SARS-CoV-2 virus can induce the development of HLA-Abs in volunteers who donate convalescent plasma (CP) after recovery from SARS-CoV-2 infection. Both HLA class I and HLA class II molecules are considered as antigens with a strong immunogenicity, and therefore, HLA-Abs directed against these antigens have the potential to initiate life-threatening transfusion-related acute lung injury (TRALI) after transfusion of blood products, such as plasma [3]. The risk of TRALI lies in the fact that CP is still widely used as an option for the treatment of COVID-19 [4]. The renowned American Food and Drug Administration (FDA) authorized the administration of CP as a new emergency investigational medication and recently updated the guidelines for the transfusion of CP [5, 6]. The main objective of the FDA revision is to make the treatment of CP more effective by considering the obligatory testing of volunteers on the presence of high titer antibodies against SARS-CoV-2 virus and the administration of only immunocompetent CP at an early stage of COVID-19 disease, due to scientific evidence that has shown that CP is most effective under these conditions [4, 7–9]. However, the updated FDA guidelines and most international regulatory agencies and professional societies do not require a mandatory testing of HLA-Abs in male and nonparous female donors, but it is mandated only in female volunteers with a history of pregnancy [10–12]. At the same time, various studies have shown that HLA-Abs can be present in healthy male, nontransplanted, nontransfused blood donors and nonparous female volunteers, and viral infections are one of the main reasons for so-called natural HLA-Abs [13–15]. On one hand, the predonation testing of volunteers on the presence of HLA-Abs might be extensive and associated with additional organizational and diagnostic challenges. On the other hand, the preformed HLA-Abs in CP have a clinical significance and they are able to trigger a TRALI in recipients. TRALI is a life-threatening complication characterized by the development of acute dyspnea and associated with noncardiogenic pulmonary edema occurring during or within few hours after the transfusion [3]. The danger of TRALI is that it is one of the most underestimated and underdiagnosed complications associated with transfusions, which often remains undetected and ends frequently fatal [16]. Therefore, TRALI is considered as one of the leading causes of mortalities related to transfusions worldwide [17]. The risk of TRALI is particularly high in the case of vulnerable COVID-19 patients, who are predisposed to develop serious and life-threatening complications due to their pulmonary system, which is already affected by SARS-CoV-2 infection. This significant threat for COVID-19 patients is recently confirmed by a published large-scale study that assessed CP transfusion-related serious adverse events in COVID-19 patients and found recipients developed severe TRALI, resulting in a high mortality rate [18]. Our hypothesis is that HLA-Abs could be the reason for the occurrence of TRALI after transfusion of CP, since it is known that SARS-CoV-2 is a strong immunogenic virus that could be associated beside autoimmunity with hyper-reaction and excessive innate and adaptive humoral immune response [19, 20]. Hence, the in vivo formation of different kinds of allo- and autoantibodies is conceivable after SARS-CoV-2 infection, including HLA-Abs. Taking all these aspects into account, we initiated this study to investigate the impact of SARS-CoV-2 virus on the building of HLA-Abs, elucidate further immunological details related to CP, and evaluate the presence of HLA-Abs in donated CP. To achieve this goal and to overcome a potential shortcoming of donated CP at our institution, all CP volunteers were screened, regardless of gender, on the presence of alloantibodies against both HLA class I and HLA class II antigens.

Materials and Methods

Study Population

A single-center prospective study was performed, between July 2020 and May 2021. Thirty-three male donors and 1 female adult donor, age between 19 and 51 years old, were enrolled. All 34 volunteers donated CP 1–3 months after recovering from symptomatic and PCR-confirmed SARS-CoV-2 infection. The donors meet eligibility criteria, including a history of COVID-19, as confirmed by approved molecular testing (nasopharyngeal and oropharyngeal swab), at least 14 days passing after the resolution of symptoms (e.g., fever, cough, shortness of breath), and a negative follow-up molecular test for SARS-CoV-2 (nasopharyngeal and oropharyngeal swab). None of the included subjects has any history of sensitization, like blood transfusions, pregnancy, or previous transplantations. All PCR tests were performed in an accredited laboratory by the College of American Pathologists (CAP). We tested a control group of 17 healthy blood donors on the presence of antibodies against HLA class I and class II molecules. All volunteers included in the control group have no history of previous SARS-CoV-2 infection or any other sensitization events, like blood transfusion, pregnancy, or transplantation.

SARS-CoV-2 Real-Time PCR

For the detection of SARS-CoV-2 virus, the Abbott Real-Time SARS-CoV-2 EUA test was used. The test, which is approved by FDA and EUA, is performed on the two platforms Abbott m2000sp and Abbott m2000rt for nucleic acid extraction and for amplification, respectively. The targeted genes are RdRp and N genes with

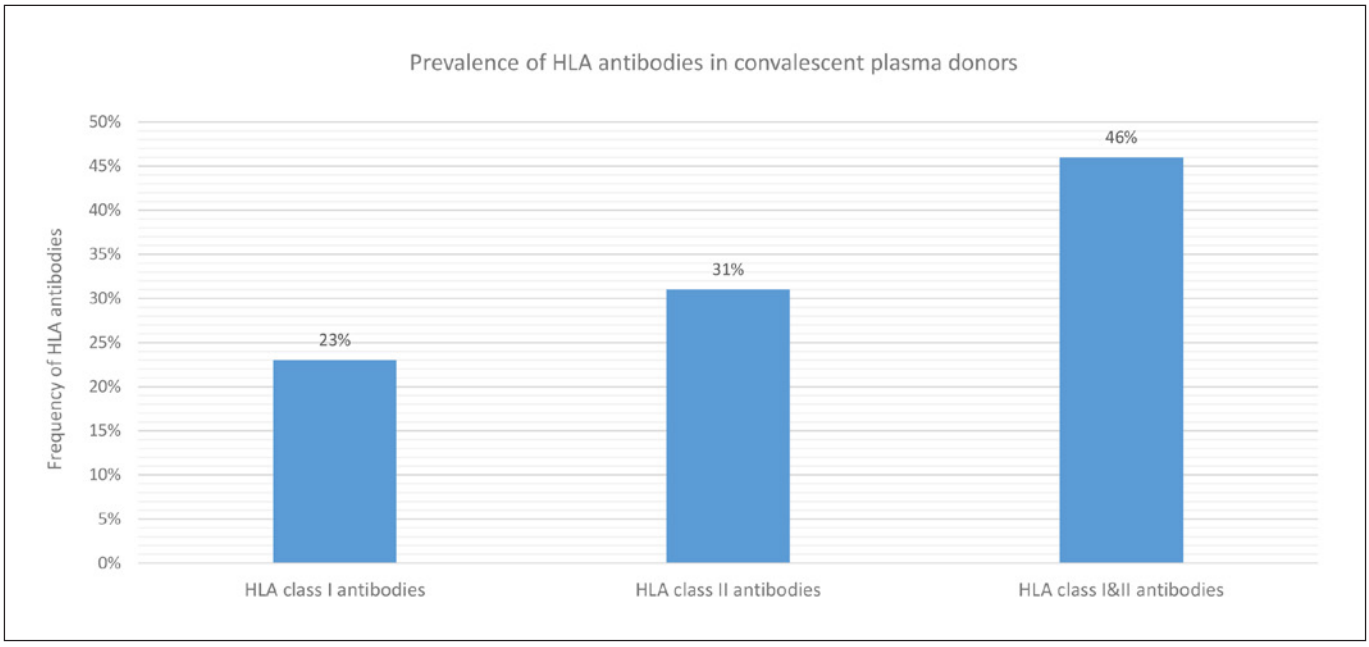

Fig. 1. Prevalence of HLA class I, HLA class II and combined HLA class I & HLA class II antibodies in convalescent plasma donors who recovered from SARS-CoV-2 infection.

a detection limit of 100 viruses c/mL. Both nasopharyngeal and oropharyngeal swabs were collected and mixed together in a normal viral transport medium. All 34 included subjects were tested positive in the PCR test and results are only considered valid if the positive and negative controls functioned properly. All molecular tests were done by experienced staff in a CAP-accredited laboratory.

HLA Antibodies

For detection of HLA-Abs, we used the flow-based bead Luminex platform technology (Luminex Corporation, Austin, TX, USA). Immucor LIFECODES LSA Single Antigen was used as a detection kit, which includes recombinant HLA molecules for all HLA-A, HLA-B, and HLA-Cw and HLA-DRB, HLA-DQB, and HLA-DPB. Serum samples were analyzed for the presence of class I and class II HLA-Abs using LIFECODES LSA Single Antigen Antibody detection kits (Immucor, Norcross, GA, USA). All tests were carried out at our HLA laboratory, which is accredited by CAP and by the American Society for Histocompatibility and Immunogenetics (ASHI). Briefly serum was centrifuged for 5 min 3,500 rpm and then 20 µL of serum incubated with 40 µL beads in dark for 30 min on a rotating platform (Heidolph Ltd., Schwabach, Germany). After 3 washes with the wash buffer, 50 µL of diluted phycoerythrin-conjugated goat antihuman IgG (Immucor) was added to the beads for 30-min incubation, suspended by 130 µL wash buffer, which was added to the wells, in the last place and positioned in the Luminex 200 system for reading. Data were analyzed using the MATCH IT database version 1. 31 (Immucor). The background values used to calculate the background adjusted mean fluorescence intensity (MFI) values were provided by the manufacturer and are derived from the average MFI of a panel of negative sera. Positive and negative control sera included in each test run generated a similar pattern to that found in the lot-specific recording sheet. Also, the bead sets include two control beads to monitor each sample performance. A positive result was classified when the MFI ≥600. The background values used to calculate the adjusted MFIs were provided by the manufacturer and are derived from the average MFI of a panel of negative sera.

Statistical Analysis

Fisher exact tests were performed to assess associations between COVID-19 disease and HLA alloimmunization by comparing the HLA-Abs results in the study with the control group with a significance threshold of an adjusted p value <0.05.

Results

We found that the majority of tested CP volunteers, who recovered form SARS-CoV-2 infection, have developed HLA-Abs (shown in Fig. 1). The antibodies were directed against both class I and class II HLA molecules. Negative HLA-Abs were found in 8 donor samples (24%). As illustrated in Figure 1, 26 out of 34 tested samples were positive either for HLA class I or for HLA class II. Six samples from these 26 positive samples were positive for class I only (23%), 8 samples were positive for class II (31%), and 12 donors found to have HLA-Abs against both class I and class II (46%). In the control group, 1 donor was

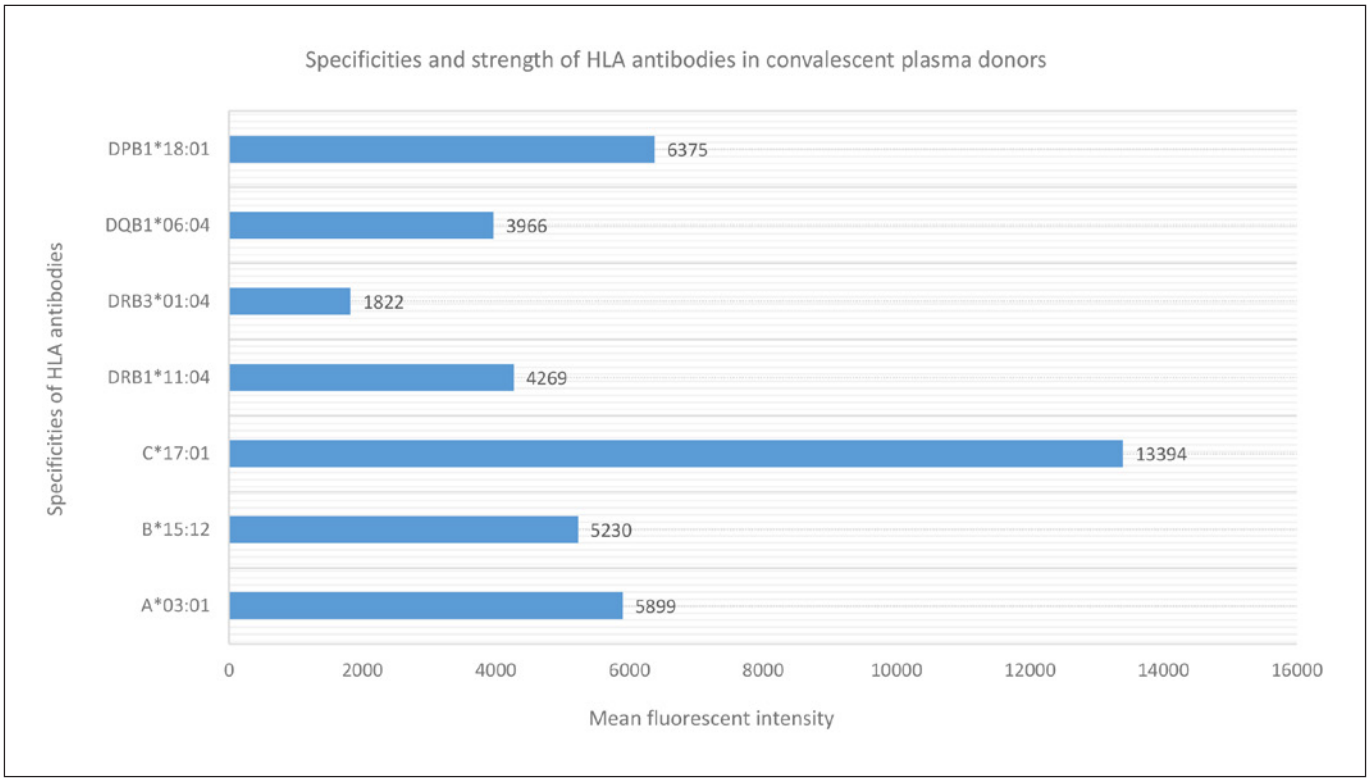

Fig. 2. HLA antibody specificities and strength given in MFI in recovered convalescent plasma donors.

Table 1. Clinical outcome of critically ill COVID-19 patients after the administration of therapeutic convalescent plasma

TCP treatment	Patient category	Time of administration of TCP	Patients received TCP, *n*	TCP volume	HLA Abs strength	SARS-CoV-2 NAT	TRALI	Outcome
Late treatment (rescue therapy)	ICU patients with multiorgan failure, advanced and incurable stage	3–4 weeks after COVID-19 diagnosis	7	200 mL	<3,000 MFI	>80	No	Death
Early treatment	ICU patients at an early stage of COVID-19 without multiorgan failure	1–3 days after COVID-19 diagnosis	20	200 mL	<3,000 MFI	>80	No	19 of 20 survived

ICU, intensive care unit.

tested positive for HLA class I antibodies only (5.8%) and 2 donors developed HLA class II antibodies (11.8%). Figure 2 shows both specificity and strength of detected HLA-Abs for both class I and class II given in MFI. A cutoff of ≥600 MFI was considered as positive. For HLA class I, the strongest HLA-Abs were formed against HLA-C*17:01 antigens (MFI 13,394), followed by antibodies directed against HLA-A*03:01 (MFI 5,899) and HLA-B*15:12 (MFI 5,023). The strongest HLA class II antibodies were HLA-DPB1*18:01 (MFI 5,375) followed by alloantibodies to HLA-DRB1*11:04 (MFI 4,269), HLA-DQB1*06:04 (MFI 3,966), and HLA-DRB3*01:04 (MFI 1,822).

The clinical outcome of the patients received TCP in our study is illustrated in Table 1. All patients received at least 200 mL TCP with a neutralization titer against SARS-CoV-2 of more than 80. Convalescent plasmas with HLA-Abs above 3,000 MFI were not considered for a transfusion. The vast majority of intensive care unit patients with early TCP administration survived, while intensive care unit patients with advanced COVID-19 and late administration of TCP were without any benefit. No confirmed TRALI cases were observed after the transfusion of TCP.

Discussion/Conclusion

Based on the presented series of volunteers enrolled in the study, who donated CP after recovery from SARS-CoV-2 infection, we found HLA-Abs in a considerable number of included donors. Overall, 76% of the cohort developed HLA-Abs. Out of these, 23%, 31%, and 46% have class I, class II, and both class I and class II, respectively. The *p* value for HLA-Abs class I and class II was below the threshold of significance (0.05) and indicates a strong evidence for the presence of HLA-Abs induced by SARS-CoV-2 virus. This statistical significance is also present when we compare the relatively high positivity rate found in our study with the positivity rate for HLA-Abs from healthy blood donors from the literature using as an example of a large Belgian study that found a positivity rate of 2% and 4% only in sera from healthy male and female blood donors, respectively [21]. In addition, the data generated from our study also support a primarily finding from MayoClinic that found a positivity rate of HLA-Abs of 10.1% of CP donors [22]. Interestingly, the positivity rate in our study was remarkably higher, which indicates a considerable link and a significant association between SARS-CoV-2 infection and the building of HLA-Abs in after SARS-CoV-2 infection in recovered CP volunteers. In this context, it is important to mention that the donors included in our study were freshly recovered from SARS-CoV-2 infection and the time interval between the resolution of COVID-19 with a negative PCR test and the collection of CP was 4 to a maximum 12 weeks, which could explain the high HLA-Abs positivity rate found in our study. The decreasing degree of the HLA-Abs titer also varies from donor to donor and can be associated with significant variances in the positivity rate [23]. Yet, the underlying immunopathological mechanisms behind the variation in the time-dependent decline of HLA-Abs are unknown. The same applies to the interindividual response and the downregulation of HLA-Abs, which can turn out very differently from one individual to another and have still remained without a satisfactory scientific explanation. Regardless of the difference in the biological changes and immunological kinetic phenomena of HLA-Abs, the high positivity rate of HLA-Abs found in the plasma of donors after recovering from SARS-CoV-2 infection is primarily an important indicator for considering HLA-Abs as a risk factor in CP transfusions and could explain the cases of deadly TRALI observed in COVID-19 patients after the administration of CP. Indeed, Joyner and colleagues [18] identified in a recently published study 21 COVID-19 patients with life-threatening TRALI directly related to CP transfusions, which corresponds to an incidence rate of 0.1%. Looking at the whole picture of preformed HLA-Abs in CP, the presented study demonstrates the close correlation between SARS-CoV-2 infection and the generation of HLA-Abs and might explain the causes behind TRALI followed the transfusion of CP in COVID-19 patients. The limitation of the informative value of our study is the relatively low number of included subjects. New efforts, e.g., large-scale studies or randomized control trials, will be able to provide more information about this clinically important finding. In conclusion, although small, our study demonstrates the impact of SARS-CoV-2 virus infection on the development of HLA-Abs against both class I and class II HLA antigens in CP volunteers. The clinical importance of this finding is to recommend the development of diagnostic strategies and prevention concepts to detect HLA-Abs prior to the transfusion of CP as a standard care. This approach has the potential to reduce the risk of TRALI and ensure safer therapeutic management of COVID-19 after CP transfusions.

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Statement of Ethics

This research project complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All participated volunteers without exception were informed about the plasma donation and the testing of blood for all parameters that mentioned in the study or relevant for donation. The study, including the consent form, was approved by the Institutional Review Board (IRB) committee of King Faisal Specialist Hospital and Research Center Jeddah with the final approval No. RC-J/467/41 and authorized by the Saudi Food and Drug Authority with the license No. 20051803. All included volunteers agreed with the study and signed the consent in a written form.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.D. initiated the study, designed the research, analyzed the results, and wrote the manuscript. K.E. who is the second author contributed equally to the first author. A.Y. analyzed the clinical results and recruited donors. R.M.B. performed SARS-CoV-2 RT-PCR tests and HLA immunoserology-sensitive assays. G.A. coordinated the study and performed SARS-CoV-2 RT-PCR tests. L.H. coordinated the study and recruited donors. G.W. involved in planning and supervising the study. S.B. involved in planning the study and prepared research documents and data for Saudi FDA approval. T.S. performed the statistical analysis. N.A. supervised the apheresis of convalescent plasma. E.C. involved in planning the study and prepared needed documents and data for Saudi FDA approval. A.N.A. planned the research, supervised the study, and analyzed the results.

Data Availability Statement

All relevant data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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